

CLAIMS

1. A method for RNA or polypeptide synthesis from a DNA template comprising the steps of

5 a) providing a cell-free system enabling RNA or polypeptide synthesis from a DNA template, said DNA template comprising a promoter with at least one UP element;

 b) recovering said synthesized RNA or polypeptide;

10 characterized in that the concentration of α subunit of RNA polymerase, but not of other subunits, is increased in said cell-free system, comparing to its natural concentration existing in the cell-free system.

2. The method according to Claim 1, wherein said system enabling RNA or polypeptide synthesis from a DNA template is a cell-free system comprising a bacterial cell-free extract.

15 3. The method according to Claim 2, wherein the promoter on the DNA template includes sequence from the *argC* gene promoter of *Bacillus stearothermophilus*, preferably, the sequence from nucleotide – 89 to +1 when the latter is the first nucleotide in mRNA of the *argC* gene.

20 4. The method according to Claim 2 or 3, wherein said cell-free system further comprises purified thermostable RNA polymerase holoenzyme.

5. The method according to Claim 4, wherein said thermostable RNA polymerase holoenzyme is from *Thermus thermophilus*.

6. The method according to any of Claims 2 to 5, wherein the concentration of α subunit of RNA polymerase is increased by adding purified α subunit of RNA polymerase to the bacterial cell-free extract.
- 5 7. The method according to Claim 6, wherein said purified α subunit is added to a final concentration comprised between 15 $\mu\text{g/ml}$ and 200 $\mu\text{g/ml}$.
8. The method according to Claim 6 or 7, wherein the cell-free extracts is prepared from cells overexpressing a gene encoding α subunit of RNA polymerase.
- 10 9. A method for the production of a protein from a DNA template in a cell-free system characterized in that it comprises the steps of
 - 15 a) providing in a reaction mixture, a bacterial cell-free system enabling the coupling of *in vitro* transcription of a specific gene from a DNA template, and the corresponding protein synthesis ;
 - b) adding to the reaction mixture the DNA template encoding the desired protein and purified α subunit of the RNA-polymerase; and,
 - 20 c) optionally, adding a thermostable RNA polymerase,
 - d) recovering the produced protein.
10. The method according to Claim 9, wherein said added thermostable RNA polymerase is from *T. thermophilus*.
11. The method according to Claim 9 or 10, wherein said purified α subunit is added to a final concentration comprised between 15 $\mu\text{g/ml}$ and 200 $\mu\text{g/ml}$.
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12. The method according to any of Claims 9 to 11, wherein a DNA-binding regulatory protein is further added to the reaction mixture at step (b).
- 5 13. The method according to any of Claims 9 to 12, wherein said DNA template comprises an amplification product of an Open Reading Frame encoding the desired protein.
- 10 14. The method according to Claim 13, wherein said DNA template further comprises an additional DNA fragment, which is at least 3 bp long, preferably longer than 100 bp and more preferably longer than 200 bp, located immediately downstream the stop codon of said Open Reading Frame.
- 15 15. The method according to Claim 13, wherein said DNA template further comprises an additional DNA fragment containing a transcriptional terminator.
- 16 16. The method according to Claim 13, wherein said transcriptional terminator is the T7 phage transcriptional terminator.
- 17 17. A reaction mixture for cell-free protein synthesis characterized in that it is prepared from cells which overexpress the gene encoding α subunit of the RNA polymerase.
- 20 18. A reaction mixture for cell-free protein synthesis characterized in that it comprises a bacterial cell-free extract and an amount of purified α subunit of RNA polymerase.
- 25 19. The reaction mixture of Claim 17 or 18, wherein said purified or overexpressed α subunit of RNA polymerase is at a concentration comprised between 15 $\mu\text{g/ml}$ and 200 $\mu\text{g/ml}$ in the reaction mixture.

20. The reaction mixture of any of Claims 17 to 19, characterized in that it further comprises a DNA template comprising a gene encoding a protein of interest under the control of a promoter with at least one UP element.
- 5 21. The reaction mixture according to Claim 17, characterized in that it further comprises a DNA-binding regulatory protein.
22. A kit for cell-free RNA and/or protein synthesis characterized in that it comprises the following components:
- a) a cell-free extract, preferably *E. coli* S30 cell-free extract;
 - 10 b) purified α subunit of RNA polymerase;
 - c) optionally, appropriate buffers and compounds for carrying out *in vitro* transcription and/or translation reaction;
 - d) optionally, amino acid mixture lacking one amino acid.
- 15 23. A kit for cell-free RNA and/or protein synthesis characterized in that it comprises the following components:
- a) a cell-free extract, preferably *E. coli* S30 cell-free extract, wherein said cell-free extract is obtained from cells overexpressing subunit of RNA polymerase;
 - 20 b) optionally, appropriate buffers and compounds for carrying out *in vitro* transcription and/or translation reaction;
 - c) optionally, amino acid mixture lacking one amino acid.
24. The kit according to Claim 22 or 23, wherein said purified or overexpressed α subunit is from *E. coli* strains.
- 25 25. Use of a purified α subunit of RNA polymerase for enhancing protein synthesis in a cell-free system.

26. The use according to Claim 25, wherein said purified α subunit of RNA polymerase is added in a cell-free system at a concentration comprised between 15 $\mu\text{g/ml}$ and 200 $\mu\text{g/ml}$.
- 5 27. The use according to Claim 26, wherein said purified α subunit of RNA polymerase is added in a cell-free system together with a thermostable RNA polymerase holoenzyme, preferably of *T. thermophilus*.